

BIOLOGY OF A POPULATION OF HOUSEFLIES, *MUSCA DOMESTICA* L.,
ON A GEOGRAPHICALLY ISOLATED RANCH FOLLOWING TWO AS
COMPARED TO THREE YEARS OF DIELDRIN SPRAYINGS

by

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INTRODUCTION

Alterations in the biology of insect populations in the laboratory, following exposure to insecticides, have been suggested by limited studies. Little has been done on the effect of repeated exposure to chlorinated insecticides to field populations of insects.

Knutson (1955) found that a laboratory strain of (Drosophila melanogaster Meigen) which survived one exposure of dieldrin in the laboratory produced 5.8 per cent more adult progeny than the controls. Afifi and Knutson (1956) reported that a laboratory strain of housefly exposed to one dieldrin treatment produced 16.7 per cent more adult progeny, their F_1 produced 69.2 per cent more adult progeny, but in the F_2 and F_3 there was no significant difference; hence, a reversion to normalcy.

The purpose of this investigation was to determine if alterations in the reproductive capacity, longevity, and fly weight do exist when chlorinated insecticide exposure is exerted gradually on a field population where natural environmental conditions occur.

MATERIALS AND METHODS

The two house fly populations used in this investigation were collected from an isolated ranch in Riley county, Kansas. The first group of flies was collected on June 21, 1956. They had survived two years of exposure to dieldrin. The first set of sprayings was in the spring, 1954, the second set of sprayings in the fall, 1954. The third set of sprayings was in the spring, 1955, and the fourth set of sprayings in the fall, 1955.

Flies collected on June 21, 1956, constituted an over-wintering

group, designated 4TP (i.e., 4 treatments, parents). (The F_3 of these parents had an LD-50 resistance to dieldrin equal to 4.14 mg./g. of flies.) A fifth spraying of dieldrin was made 15 days after the overwintering (4TP) collection, on July 6, 1956, during which all possible breeding places were sprayed with a mixture of 180 g. of 50 per cent dieldrin wettable powder dissolved in five gallons of water; a total of 25 gallons of the insecticide was used. Observations five days after spraying indicated a great reduction in the fly population. Flies which survived this fifth spraying were collected 33 days after spraying, on August 8, 1956. This group was designated 5TP (i.e., 5 treatments, parents). (The F_1 of these parents had an LD-50 resistance to dieldrin equal to 96.98 mg./g. of flies.) Approximately the same number of both groups (2,300 females) was collected for laboratory studies.

The investigation was conducted in an insecticide-free room with a constant temperature of $80^{\circ}\text{F.} \pm 2^{\circ}$. The flies of each group were brought into the room immediately following collection and separated from other species, using the CO_2 method outlined below. On return of the two parent groups to the cages, they were supplied with food, consisting of powdered milk and granulated sugar, and with water until enough eggs were collected, as described below, to produce mass numbers of the F_1 generations. Eggs collected for increase from each of the parent groups were placed in twelve 10 pound, waxed paper cheese tubs, five-tenths ml. (approximately 3,222 eggs) per tub. One package of yeast, $1\frac{1}{2}$ oz of dimalt, 36 oz. of water, and 1,600 ml. dry CSMA fly larval media were mixed and placed with the eggs in tubs, and covered with cheese cloth held in place with a large rubber band.

After eight days, the resulting pupae were separated and placed in cages to emerge. Cages were the same type as described by Ouye (1957).

Twenty-four hours after the flies emerged, they were transferred by a moving air stream into one quart cylindrical cardboard cartons with screened ends in preparation for sexing. Carbon dioxide anaesthesia was used to facilitate handling and sexing. The use of CO₂ anaesthesia was kept at a minimum, well within the limits indicated by Williams (1946).

Five hundred males and 500 females were transferred into each cage. A total of 13 cages were used for the 4TF₁ flies and 11 cages for the 5TF₁ flies.

Food and water were changed daily. The food consisted of a mixture of one volume granulated sugar to two volumes of powdered milk. Oviposition site and water source consisted of crystallization dishes, 70 mm. in diameter by 50 mm. high, in which cork covered with muslin six inches by six inches was floated in water.

Longevity was determined by making a daily record of the number of dead F₁ male and female flies. They were dragged out of the cage with a looped wire as the front glass panel was raised high enough to permit entry of the wire.

To record egg production in each cage, the eggs which had been oviposited on muslin were washed off with a stream of water from a polyethylene wash bottle into a 25 ml. beaker. The eggs were concentrated by withdrawing the excess water with a pipette. The eggs were then transferred to a 15 ml. graduated centrifuge tube and measured volumetrically. The volume of eggs was converted to number of eggs since each 0.3 ml. equals 2,000 eggs \pm 180. When eggs were too few to measure volumetrically, the eggs were counted and the exact number recorded.

After egg counts from each cage were recorded each day, the eggs from all replicates were thoroughly mixed. To determine hatchability, moistened

filter paper was fitted into each of three petri dishes. Two hundred eggs from the combined collection were placed in each dish in groups of ten eggs to facilitate counting. The filter papers were kept saturated by adding water at intervals. Records of hatched and unhatched eggs were made at 24 and 48 hours.

To determine pupation and emergence, approximately 2,000 eggs were taken from the same batch of mixed eggs used in the hatchability studies. These were planted daily in standard CSMA fly larva media in ten pound waxed paper cheese tubs. Eight days following planting of each daily batch of eggs, the pupae were separated and counted and then placed in cages without food and water. After all flies had emerged and died, the number of emerged flies were sexed and recorded. In all cases some of the larvae had not pupated at the end of eight days; these larvae were held until all pupation had occurred.

For weight studies, these resulting adult progeny (F_2) were placed in petri dishes and desiccated at 100°C. for 72 hours in a hot air oven. The petri dishes were then placed in calcium chloride desiccating containers. Subsequently, the flies and dishes were weighed with a Christian Becker chainomatic balance. The dishes with flies were then replaced in the hot air oven and the process repeated, this time for 12 hours, to derive a constant weight. Replications could not be made due to the small number of progeny.

Data were based on the first 28 days following initial oviposition because egg production was almost negligible thereafter.

For statistical analysis of egg production, the number of eggs from each replicate was cumulated each successive day. Eggs of both groups were analyzed for significance by the Wilcoxon (1945) and Mann and Whitney (1947) ranking test. A similar analysis was made for female fly days (the cumulative number of females remaining alive each day) and for average number

of eggs per female fly day.

Hatchability, pupation and emergence were analyzed for significance by chi-squaring the weekly totals and the four week summary. A similar procedure was followed for analyzing calculated adult progeny.

In the weight studies, each day's progeny of the two groups was analyzed for significance by "Student's t-test".

For the above studies a 5 per cent level of significance was used.

RESULTS AND CONCLUSIONS

Egg Production

Table 1 shows that the cumulative egg production during the first four days was equal for both groups. From the fifth through the tenth day, with the exception of the eighth day where production was approximately equal, the LT egg production was greater than the ST. Between the eleventh and the twenty-eighth day the egg production was approximately equal except for the sixteenth and seventeenth days when it was equal. By the end of 28 days the LT group had produced 1,808,353 eggs and the ST group 1,379,836, statistical analysis indicating the LT to be approximately equal to that of the ST. Evaluation of these figures must take into consideration the fact that the LT group represented eggs from 6,500 females (13 replicates) and the ST group eggs from 5,500 females (11 replicates). Converted, comparable figures would be 1,530,144 and 1,379,836 as 10.9 per cent greater number of eggs in the LT.

Longevity (Female Fly Days)

These data were obtained by cumulating the totals of F₁ females remaining alive each day (Table 2). Although the LT flies ranked somewhat higher each day than the ST flies, statistical analysis indicated that female fly

Table 1. Cumulative total egg production of 13 replicates of 500 females each, collected following 4 dieldrin treatments (4T); and 11 replicates following 5 dieldrin treatments (5T). Egg counts made on successive days following initial oviposition. The 4th treatment applied in fall but collections made the following spring. 5th treatment applied to same population during mid-summer.

Successive days	Cumulative total eggs		Yo	Conclusion
	4T	5T		
1	30,664	181	0.43	4T = 5T
2	39,422	6,679	0.96	"
3	100,108	170,880	-1.33	"
4	204,226	171,907	0.09	"
5	366,884	187,090	2.00	4T > 5T
6	487,542	269,208	2.06	"
7	556,936	293,679	2.06	"
8	639,561	439,671	1.42	4T \approx 5T
9	856,889	551,663	2.06	4T > 5T
10	970,215	602,479	2.29	"
11	1,126,871	814,473	1.30	4T \approx 5T
12	1,231,525	866,768	1.54	"
13	1,359,515	988,098	1.54	"
14	1,436,746	1,048,305	1.36	"
15	1,457,671	1,084,652	1.13	"
16	1,498,373	1,150,801	0.90	4T = 5T
17	1,565,789	1,204,387	0.96	"
18	1,618,603	1,227,647	1.01	4T \approx 5T
19	1,697,931	1,266,922	1.13	"
20	1,706,751	1,306,695	0.90	"
21	1,749,602	1,339,379	1.07	"
22	1,758,989	1,349,674	1.19	"
23	1,785,952	1,362,186	1.30	"
24	1,787,519	1,369,449	1.19	"
25	1,793,011	1,374,311	1.19	"
26	1,802,098	1,374,985	1.30	"
27	1,802,801	1,377,383	1.25	"
28	1,808,353(a)	1,379,836	1.30	"

(a) converted total to compensate for differences in number of replications is 1,530,144.

Table 2. Cumulative female fly days of 13 replicates of 500 females each, collected following 4 dieldrin treatments (4T); and 11 replicates of dieldrin treatments (5T) of counts made on successive days following initial oviposition.

Successive days	Cumulative total eggs		Yo	Conclusion
	4T	5T		
1	5,722	4,633	1.25	4T \approx 5T
2	11,374	9,211	1.19	"
3	16,962	13,721	1.25	"
4	22,490	18,168	1.19	"
5	27,945	22,552	1.19	"
6	33,331	26,876	1.19	"
7	38,657	31,133	1.30	"
8	43,897	35,294	1.42	"
9	48,990	39,365	1.42	"
10	53,959	43,323	1.30	"
11	58,762	47,103	1.36	"
12	63,430	50,740	1.36	"
13	67,867	54,213	1.36	"
14	71,972	57,512	1.30	"
15	75,896	60,608	1.48	"
16	79,533	63,446	1.48	"
17	82,856	66,083	1.59	"
18	85,898	68,484	1.48	"
19	88,547	70,674	1.48	"
20	90,939	72,607	1.42	"
21	93,066	74,302	1.36	"
22	94,966	75,826	1.25	"
23	96,533	77,086	1.30	"
24	97,958	78,136	1.25	"
25	99,180	79,007	1.25	"
26	100,214	79,727	1.19	"
27	101,110	80,296	1.07	"
28	101,836(a)	80,763	1.01	"

(a) converted total to compensate for differences in number of replications is 86,169.

days for the 4T group was approximately equal to that of the 5T group. Evaluation of the actual number of female fly days must again take into consideration the number of replications for each group, since the 4T consisted of 6,500 females and the 5T of 5,500 females. Corrected, comparable figures would be 86,169 and 80,763, a 6.7 per cent increase in the 4T.

Average Number of Eggs per Female Fly Day

To obtain data in Table 3, the cumulated daily totals in Table 1 were divided by the corresponding F₁ female fly days in Table 2. Between the fifth and tenth day, with the exception of the eighth day when both groups were approximately equal, there was a tendency for the 4T flies to average more eggs per female fly day than the 5T flies. Prior to this period, and thereafter through the twenty-eighth day, both groups were approximately equal. This is essentially the same trend that was found in Table 1, and would be expected, since the cumulative female fly days was approximately equal throughout the life span of both groups.

Hatchability, Pupation and Emergence

Data presented in Table 4 were based on the actual number of eggs hatched from an aliquot of 600 eggs per day, and the number of pupations and adult emergences from an aliquot of 2,000 eggs per day, except as noted in the table.

In all three instances, i.e., hatchability, pupation and emergence, the 4T group underwent a greater survival rate than the 5T group during the first week.

During the second week, hatchability in both groups was approximately equal. In pupation and emergence, like the first week, the 4T group surpassed the 5T.

Table 3. Cumulative average number of eggs per fly by days of 13 replicates of 500 females each, collected following 4 dieldrin treatments (4T) and 11 replicates of 5 dieldrin treatments (5T) on successive days following initial oviposition.

Successive days	:	Average number of eggs per fly		:	Yo	:	Conclusion
	:	4T	5T	:		:	
1		5.4	0.0		0.90		4T \approx 5T
2		3.5	0.7		1.51		"
3		5.9	12.5		-1.45		"
4		9.1	9.5		0.03		"
5		13.1	8.3		1.83		4T > 5T
6		14.6	10.0		2.09		"
7		14.4	9.4		1.88		"
8		14.6	12.5		1.13		4T \approx 5T
9		17.5	14.0		1.91		4T > 5T
10		18.0	13.9		2.00		"
11		19.2	17.3		1.10		4T \approx 5T
12		19.4	17.1		1.33		"
13		20.0	18.2		0.87		"
14		20.0	18.2		1.04		"
15		19.2	17.9		0.90		"
16		18.8	18.1		0.61		"
17		18.9	18.2		0.61		"
18		18.8	17.9		0.78		"
19		19.2	17.4		0.84		"
20		18.8	18.0		0.72		"
21		18.8	18.0		0.61		"
22		18.5	17.8		0.58		"
23		18.5	17.7		0.72		"
24		18.2	17.5		0.70		"
25		18.1	17.4		0.70		"
26		18.0	17.2		0.72		"
27		17.8	17.2		0.61		"
28		17.8	17.1		0.70		"

Table 4a. Weekly totals and four week total of hatchability, pupation and emergence of eggs produced by flies surviving 4 dieldrin treatments (4T) and 5 dieldrin treatments (5T).

Week		Total eggs	Hatchability		Chi- square	Conclusion (with one degree of freedom)
			Hatched	Percent		
1	4T	4,200	3,736	89.0	120.02	4T > 5T
	5T	3,600	2,881	80.0		
2	4T	4,200	3,184	75.8	0.85	4T \approx 5T
	5T	4,200	3,220	76.7		
3	4T	4,000	2,568	64.2	2.79	4T \approx 5T
	5T	4,200	2,771	66.0		
4	4T	4,200	2,467	58.7	68.46	5T > 4T
	5T	3,600	2,442	67.8		
Total	4T	16,600	11,955	72.0	1.04	4T \approx 5T
	5T	15,600	11,314	72.5		

Table 4b.

Week		Total eggs	Pupation		Chi- square	Conclusion (with one degree of freedom)
			Pupated	Percent		
1	4T	14,533	3,493	24.0	188.49	4T > 5T
	5T	10,000	1,674	16.7		
2	4T	14,466	2,542	17.6	7.74	4T > 5T
	5T	14,000	2,287	16.3		
3	4T	14,000	2,588	18.5	57.05	4T > 5T
	5T	14,000	2,115	15.1		
4	4T	10,000	2,349	23.5	317.85	4T > 5T
	5T	8,000	1,042	13.0		
Total	4T	52,999	10,972	20.7	447.92	4T > 5T
	5T	46,000	7,118	15.5		

Table 4c.

<u>Emergence</u>						
Week	:	Total	:	Adults	:	Chi
	:	eggs	:		:	square
	:		:		:	(with one degree
	:		:		:	of freedom)
1	LT	14,533		2,583	17.8	771.95
	ST	10,000		570	5.7	
2	LT	14,466		2,093	14.5	116.88
	ST	14,000		1,434	10.2	
3	LT	14,000		2,109	15.1	349.30
	ST	14,000		1,110	7.9	
4	LT	10,000		1,855	18.6	481.92
	ST	8,000		582	7.3	
Total	LT	52,999		8,640	16.3	1,533.76
	ST	46,000		3,696	8.0	

During the third week, hatchability in both groups was approximately equal. Again, the 4T group surpassed the 5T group in both number of pupae and adults.

Hatchability during the fourth week reversed in trend so that the 5T group was superior to the 4T group. As in the previous three weeks the 4T group surpassed the 5T group in both pupation and emergence.

Totaling for the entire four weeks, hatchability of both groups was approximately equal, but pupal attainment and adult emergence in the 4T group exceeded that of the 5T group.

Table 5 presents certain data in Table 4 in terms of per cent mortality, also based upon rates of survival between each successive life history stage, as well as upon a constant number of eggs. The 4T group underwent a slightly greater mortality in the egg stage (0.5 per cent), but the 5T group underwent a greater mortality in both the larva to pupa stage (5.7 per cent) and pupa to adult stage (3.1 per cent). A comparison of the overall difference in survival from egg to adult shows that the 5T group underwent a significantly greater mortality (8.3 per cent) than the 4T, indicating that the losses suffered by the 5T group between the larva and pupa and between the pupa and adult greatly exceeded the gain between the egg and larva.

Calculated Total Adult Progeny

While the data on adult emergence in Table 4 are based upon results of a standard number of eggs in both the 4T and 5T groups (viz., emergence tests), Table 6 combines these data, i.e., per cent adult emergence, with the actual eggs produced (Table 1), to produce the calculated number of adult progeny to have been expected.

Table 5. Mortality among life history stages, during first four weeks, of progeny of flies surviving 4 dieldrin treatments (4T) and 5 dieldrin treatments (5T).

Egg to larva : Larva to pupa : Pupa to adult : Egg to adult				
4T	28.0	51.3	4.4	83.7
5T	27.5	57.0	7.5	92.0
% difference (4T vs. 5T)	-0.5	+5.7	+3.1	+8.3

Table 6. Calculated weekly totals and four week total of the number of potential adult progeny based on actual number of eggs produced per day (Table 1) and corresponding emergence rate (Table 4).

Week	:	Total	:	Percent	:	Potential	:	Conclusion
	:	eggs	:	emergence	:	adults	:	(with one degree
	:	(Table 1)	:	(Table 4)	:		:	of freedom)
1	4T	556,936		17.8		99,135(a)		4T > 5T
	5T	293,679		5.7		16,740		
2	4T	879,810		14.5		127,572(a)		4T > 5T
	5T	754,626		10.2		76,972		
3	4T	312,856		15.1		47,241(a)		4T > 5T
	5T	291,074		7.9		22,995		
4	4T	58,751		18.6		10,928(a)		4T > 5T
	5T	40,457		7.3		2,953		
Total	4T	1,808,353				284,876(a)		4T > 5T
	5T	1,379,836				119,660		

(a) converted total to compensate for differences in number of replications is: 1st week, 83,884; 2nd week, 107,945; 3rd week, 39,973; 4th week, 9,247; total, 241,049.

During each of the four weeks the hT group exceeded the ST group, the total at the end of the four weeks resulted in the hT group producing a calculated 25.9 per cent greater number of progeny than the ST. (Converted, comparable numbers of adults are 249,413 and 110,387, to allow for differences in number of replications.) This difference in progeny was the result of a combination of greater egg production of the hT group, especially during the first two weeks, and a lower mortality in pupation and emergence rates during all four weeks (Table 4).

Weights

Table 7 shows the daily mean weights of the male and female adult progeny of the F₁ in both groups. The mean weight for twenty-two days, when weights could be compared, of the hT group (3.4 mg. for males, 3.8 mg. for females) in both sexes was less than the ST group (3.7 mg. and 4.0 mg., respectively).

Table 7. Weight (in mg.) of adult male and female progeny. 4 dieldrin treatments (4T) and 5 dieldrin treatments (5T).

Successive days	Mean weight		Mean weight	
	females		males	
	4T	5T	4T	5T
1	3.6	---	3.2	---
2	3.9	4.5	3.4	4.0
3	4.1	4.0	3.7	3.6
4	4.2	---	3.7	---
5	3.9	4.2	3.4	3.8
6	3.9	3.9	3.5	3.8
7	3.7	4.1	3.3	3.8
8	3.8	4.2	3.4	3.8
9	4.1	4.2	3.7	3.7
10	4.1	4.1	3.7	3.8
11	3.9	4.0	3.4	3.7
12	3.7	3.7	3.3	3.5
13	3.8	3.9	3.3	3.6
14	3.8	3.8	3.5	3.5
15	3.8	4.2	3.4	3.6
16	3.5	3.8	3.2	3.6
17	3.8	4.1	3.4	3.8
18	3.6	3.4	3.2	3.4
19	3.9	3.9	3.5	3.5
20	3.7	4.0	3.2	3.6
21	3.9	4.0	3.5	3.7
22	3.6	4.2	3.2	3.8
23	3.8	4.0	3.4	3.6
24	---	4.0	---	3.6
25	3.8	3.7	3.4	3.4
26	3.7	---	3.2	---
27	---	---	---	---
28	3.8	---	3.6	---
Mean for 22 days(a)	3.8	4.0	3.4	3.7

Females: $t = 5.00$; 21 Degrees of freedom; Conclusion - 5T > 4T.

Males: $t = 7.50$; 21 Degrees of freedom; Conclusion - 5T > 4T.

(a) The reason for the absence of certain daily means is due to the lack of sufficient eggs laid on those respective days to obtain a sufficient sample.

SUMMARY

On a cumulative daily total basis the 4T flies generally outlayed the 5T flies during the early part of their productive life resulting in a 10.9 per cent greater number of eggs from the 4T.

Although longevity of the 4T flies was somewhat greater each day than the 5T flies, statistical analysis indicated them to be approximately equal. For the 28 day period there was a 6.7 per cent increase in the 4T.

In cumulative average number of eggs per female fly day at the end of 28 days the 4T group averaged 17.8 and the 5T group 17.1.

For the four week period hatchability was approximately equal for both groups, but in pupation and emergence the 4T flies exceeded the 5T flies. A comparison of the overall difference in survival from egg to adult shows that the 5T group underwent a significantly greater mortality (8.3 per cent) than the 4T.

The calculated total adult progeny at the end of four weeks resulted in the 4T group producing a calculated 125.9 per cent greater number than the 5T.

The daily mean weight of the 4T group in both sexes was less than the 5T group.

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1957

Laboratory studies of houseflies collected following field sprayings with dieldrin were undertaken to determine if alterations in the reproductive capacity, longevity, and fly weight occurred. This study involved a comparison of flies collected in the spring following four sprayings of dieldrin over a period of the previous 2 years (4T) and flies collected following a 5th spraying during midsummer of the third year (5T).

Eggs were collected from field-collected flies and reared to produce the F₁ generation for increase. After emergence, 500 males and 500 females were transferred into each cage. A total of 13 cages were used for the 4TF₁ flies and 11 cages for the 5TF₁ flies.

Number of eggs produced, number of parent females surviving, number of eggs hatched, resulting pupation, and resulting emergence were recorded daily. All data were statistically analyzed for significance.

On a cumulative daily total basis the 4T flies generally outlayed the 5T flies during the early part of their productive life. Comparable figures for the twenty-eight day period were 1,530,144 eggs for the 4T groups and 1,379,836 for the 5T, a 10.9 per cent greater number of eggs from the 4T.

Although longevity of the 4T flies was somewhat greater each day than the 5T flies, statistical analysis indicated them to be approximately equal. Comparable figures for 28 days were 86,169 female fly days for the 4T group and 80,763 for the 5T, a 6.7 per cent increase in the 4T.

The cumulative average number of eggs per female fly day in both groups followed the same trend as in egg production, which would be expected since the cumulative female fly days were approximately equal in both groups throughout their life span. At the end of 28 days the 4T group averaged 17.8 and the 5T group 17.1.

The 4T flies produced eggs with a greater percentage of hatchability

during the first week and the 5T flies a greater percentage during the fourth week. Hatchability was approximately equal during the second and third weeks, and for the four week total. Pupation and emergence was consistently greater in the 4T flies during the four week period. The 4T group underwent a slightly greater mortality in the egg stage (0.5 per cent), but the 5T group underwent a greater mortality in both the larva to pupa stage (5.7 per cent) and pupa to adult stage (3.1 per cent). A comparison of the overall difference in survival from egg to adult shows that the 5T group underwent a significantly greater mortality (8.3 per cent) than the 4T, indicating that the losses suffered by the 5T group between the larva and pupa and between the pupa and adult greatly exceeded the gain between the egg and larva.

The calculated total adult progeny of the 4T group exceeded that of the 5T group during each of the four weeks. The total at the end of four weeks resulted in the 4T group producing a calculated 25.9 per cent greater number than the 5T. This was the result of the greater egg production of the 4T group, especially during the first two weeks, and the greater mortality of the 5T group in pupation and emergence during all four weeks.

The daily mean weight of the 4T group (3.4 mg. for males, 3.8 mg. for females) in both sexes was less than the 5T group (3.7 mg. and 4.0 mg., respectively).

Date Due

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